HIV and other Retroviruses

Introduction

Within DASTLR, research involving human immunodeficiency virus (HIV) and other retroviruses is conducted in two branches, the HIV and Retrovirology Branch and the HIV Immunology and Diagnostics Branch. The mission of the HIV/AIDS and Retrovirology Branch is to investigate HIV and other human and zoonotic retroviruses, including the diseases they cause, their modes of transmission, and the means for their control through virus detection, isolation, and characterization by virologic, molecular, and cellular biologic methods. The Branch is divided into four sections -- Viral Evolution and Transmission Section; Retroviral Genetics Section; Molecular Epidemiology and Zoonoses Section; and Virology Section -- which interact extensively through working groups charged with specific program responsibilities. These working groups are currently focused on collaborative HIV vaccine studies, detection and characterization of HIV variants, surveillance for zoonotic retroviruses, detection and surveillance of drug-resistant HIV, and mucosal-HIV interactions.

The HIV Immunology and Diagnostics Branch, DASTLR, conducts basic and applied studies of microbial-host interactions that occur in infections, particularly infection with HIV. Studies are conducted on diagnostics, natural history, mechanisms of infection, immunopathogenesis, and the biology of host-microbe interaction. These studies are conducted to improve diagnostic accuracy, to analyze and distinguish effective versus deleterious immune responses, and to identify targets for immune intervention. Branch activities involve a) developing, evaluating, and improving assay procedures for immune mechanisms and diagnosis of diseases; b) performing diagnostic testing for laboratories and organizations within NCID and CDC as well as outside organizations; and c) performing or collaborating in the performance of clinical, epidemiologic, and field studies of immunologic disease states. The Branch comprises seven laboratories: Clinical Immunology, Immunoregulation, Immune Response, Immunopathogenesis, Immunogenetics, Molecular Immunology, and HIV Diagnostics.

1999 Accomplishments

HIV Biology, Molecular Epidemiology, and Development of New Surveillance Markers

- In collaboration with the Prevention Services Research Branch, Division of HIV/AIDS–Surveillance and Epidemiology (DHAP-SE), NCHSTP,

  --investigated the genetic divergence among HIV-1 subtype B viruses isolated from newly diagnosed patients in ten U.S. cities. A broad diversity among currently circulating HIV-1 subtype B variants was documented. These sequences provide important information for developing and updating diagnostic assays and for current vaccine research. After sero-incidence testing for detection of incident strains, we will conduct genetic analysis of these viral sequences to obtain critical information for evaluating breakthrough infections in persons immunized by subtype B vaccines.

--continued an extensive domestic survey of newly HIV-infected persons to determine whether people in the United States are infected with HIV-1 subtypes other than subtype B (non-B). Recent data indicate that non-B subtypes (subtypes A, F, and G) comprised 1.6% of the 542 HIV-1 infections studied. While most of these non-B infections were found in persons born in other, predominantly African, countries, secondary transmissions have occurred within the United States.

- Validated an HIV-2/simian immunodeficiency virus (SIV) viral quantitation assay. Established a collaborative agreement with Roche Molecular Systems to exchange reagents and further explore variability in test sensitivity for detection of field isolates.

- In collaboration with the Department of Pathology, Emory University, explored parameters of disease progression and outcome in a *Macaca nemestrina* model of human retroviral (HIV-2) infection. Correlated HIV-2 viral load during disease development with other indicators of immune suppression.

- Developed a highly successful vaginal mucosa challenge protocol in the *Macaca nemestrina* model of human retroviral (HIV-2) infection to study vaginal mucosal exposure and transmission of HIV. In collaboration with NCHSTP/DHAP-SE scientists, concluded a study which represents the first demonstration that post-exposure prophylaxis after sexual exposure to a human-derived retrovirus can effectively prevent transmission.

- Developed alternative and complementary approaches to study the HIV mutation rate from patient material and to correlate viral mutation rate with HIV disease outcome and progression.

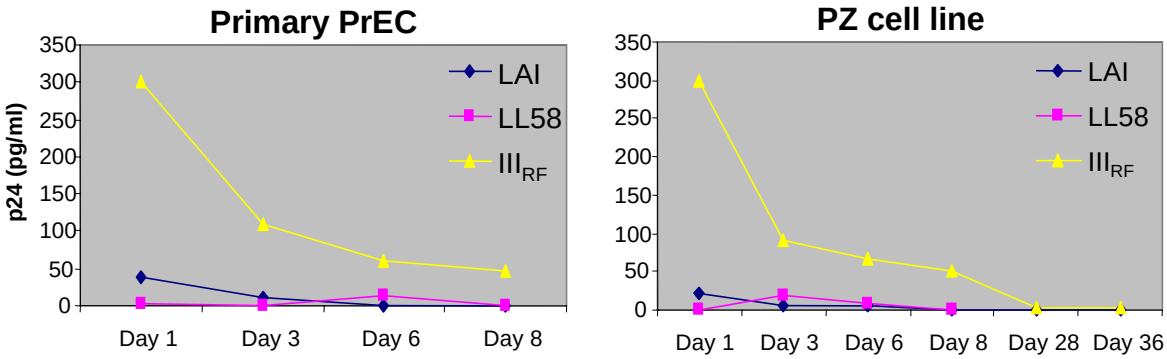
Factors Affecting HIV Replication/AIDS Progression: Host Factors, Coreceptors, and Opportunistic Infections

- Continued studies to determine if copathogens commonly found in AIDS patients modulate HIV replication. Provided evidence that common pathogens, such as *Cryptococcus neoformans, Mycobacterium avium,* and *Mycobacterium tuberculosis,* can induce HIV replication, although through different mechanisms. Understanding these various pathways could help in the development of better therapeutic regimens to control HIV replication.
- In collaboration with DHAP-SE/NCHSTP, continued to characterize the production of a novel macrophage-dependent factor(s) that can suppress HIV replication from an integrated provirus. This factor may be related to protection in highly exposed individuals.

- Further improved a novel magnetic bead method for identifying the cellular source of HIV replication in patient samples and demonstrated the impact of opportunistic diseases and cervical lesions in altering the cellular origin of HIV replication.

- In collaboration with the Bacterial STD Branch (BSTD), DASTLR, examined the impact of ulcerative and non-ulcerative STDs, as well as hormone levels, in the epithelial cell model system. These studies will provide insight on factors that may influence HIV shedding and susceptibility. Prepared manuscript describing the development of an epithelial cell model system which mimics HIV sexual transmission routes for use in a study of factors that may affect the transmissibility of HIV. Thus far, data have shown that the inability to productively infect primary epithelial cells is due to lack of coreceptor/receptor expression.

Inability to Productively Infect Epithelial Cells



	Day 1	Day 3	Day 6	Day 8	Day 28	Day 36
LAI	-	nd	nd	-	-	-
III$_{RF}$	+++	nd	nd	-	++	++	-

+++= >5000 copies; ++= 5000 - 500 copies

Neither cell-free (LAI) nor cell-associated (III$_{RF}$) HIV-1 was able to infect primary epithelial cells or cell lines. Further analysis showed these urogenital epithelial cells lack the HIV-1 receptor (CD4) and coreceptors (X4 and R5). These data show that urogenital epithelial cells are not an infected reservoir for HIV-1 transmission.

- Using the magnetic bead-capture protocol, detected unique virus-host immune response interactions during the acute phase of HIV infection. These may reflect HIV replication in non-activated cells of the immune system at the point of viral entry or rapid clearance of circulating viruses that incorporate HLA-related molecules during the process of immune maturation.

- Through a collaboration with Tularik, Inc., evaluated a panel of novel inhibitors that target cellular pathways involved in HIV transcription and identified new cellular components essential for HIV replication as possible therapeutic targets.

Emory Vaginal Ecology Study

- Continued prospective analysis of genital tract virus in HIV-1-infected women. Clinical and virologic data are now available on more than 40 women who have been enrolled in

the study for longer than 2 years and who have had semi-annual exams. A longitudinal analysis examining the relationship between viral loads in peripheral blood and genital tract with genital tract infections, immune status, and disease progression is ongoing.

- Determined that the level of ongoing virus replication in HIV-1-infected cells in genital secretions is correlated with the level of cell-free virus in the female genital tract. This suggests that most of the cell-free HIV-1 in female genital secretions is produced in cells within the genital tract.

- Examined changes in genital tract virus load and proinflammatory cytokines during the menstrual cycle. Determined that weekly changes in vaginal virus load are correlated with changes in the level of the proinflammatory cytokine IL-1b in genital secretions. Two other proinflammatory cytokines, TNF-alpha and IL-6, were also found in the genital secretions of these women, but at a much lower rate, and their presence was not concordant with the presence of cell-free HIV.

- Discovered that in the presence of genital tract inflammation HIV-1 in genital secretions has a much higher concentration of HLA-DR on the virion surface compared to that in blood plasma. The presence of increased levels of HLA-DR on the virion surface has been shown to enhance viral infectivity.

- Developed a protocol, using the Amp-RT assay, to detect and quantify replication competent HIV-1 in female genital secretions.

- Established a protocol that allows for the study of HIV-1 replication kinetics in the female genital tract. Preliminary results suggest a much slower rate of HIV-1 replication in the female genital tract compared to that in peripheral blood.

- Initiated a project to determine the cellular origin of HIV-1 in the female genital tract using in situ localization of HIV-1 RNA.

HIV Drug-Resistance Studies

- Completed and published the results of two studies on the development of rapid, nonculture-based phenotypic assays for measuring resistance to 3TC and nevirapine. These methods analyze the susceptibility of plasma reverse transcriptase (RT) to RT inhibitors by using the Amp-RT assay.

- In collaboration with the Hospital Infections Program (HIP), NCID, determined prevalence of drug resistance among HIV-infected source patients of occupational needlestick exposures. This activity will assess the prevalence of drug resistance in this population and help determine whether resistance testing of source patients might be useful in algorithms for choice of post-exposure prophylaxis.

- In collaboration with Virco, and ViroLogic, initiated a study to compare the commercially available phenotypic assays provided by these companies. This study will help us determine the most appropriate assay for conducting phenotypic analysis of samples from various CDC studies.

- Assisted NCHSTP-DHAP-SE epidemiologists in determining prevalence of drug resistance in persons recently infected with HIV-1 and in infected pregnant women receiving ZDV-containing regimens.

- Investigated the mechanisms of acquisition of the multi-drug resistance pathway mediated by the Q151M mutation and identified a specific polymorphism in the virus that is required for developing this pathway. Data may have implications for policy regarding drug resistance testing before initiation of antiretroviral therapy.

- Developed HIV isolation, expansion, and titration protocols for samples from patients who had genotypic evidence of drug-resistant virus. Provided stock cultures to contractor (Southern Research, Inc.) for drug-sensitivity phenotyping.

- Identified the differential cellular compartmentalization of drug-resistant HIV phenotypes within T-cells or macrophages of an infected individual, and applied this information to the study of perinatal transmission and the effect of long-lived reservoirs of resistant virus upon transmission.

Human T Lymphotropic Virus (HTLV) Surveillance and Disease Associations

- In collaboration with investigators at FDA, the National Heart, Lung, and Blood Institute (NHLBI) at the National Institutes of Health (NIH), and New York University, published the results of a blinded testing for tax gene sequences on a panel of samples from Maryland blood donors who tested seronegative with licensed HTLV screening assays. No tax sequences could be amplified from these samples.

- In collaboration with investigators at the University of Washington and in Peru, continued studying the presence of HTLV-infected cells in vaginal swabs which correlate with the presence of multiple STDs. These data suggest that STDs may be involved in HTLV shedding.

- In collaboration with the Georgia Institute of Technology, conducted functional analysis of the HTLV pol gene to understand the transmission of HTLV. A comparative analysis of HTLV/HIV RT/Pol and the ratio of RT to packaged genomic viral RNA is underway to better understand the differences in the transmission efficiency of HIV compared with HTLV.

- In collaboration with investigators from the Retrovirus Epidemiology and Donor Study (REDS), completed and published the results of a study on the absence of divergent

simian T lymphotropic virus (STLV) infection in U.S. blood donors with HTLV indeterminate serologic results.

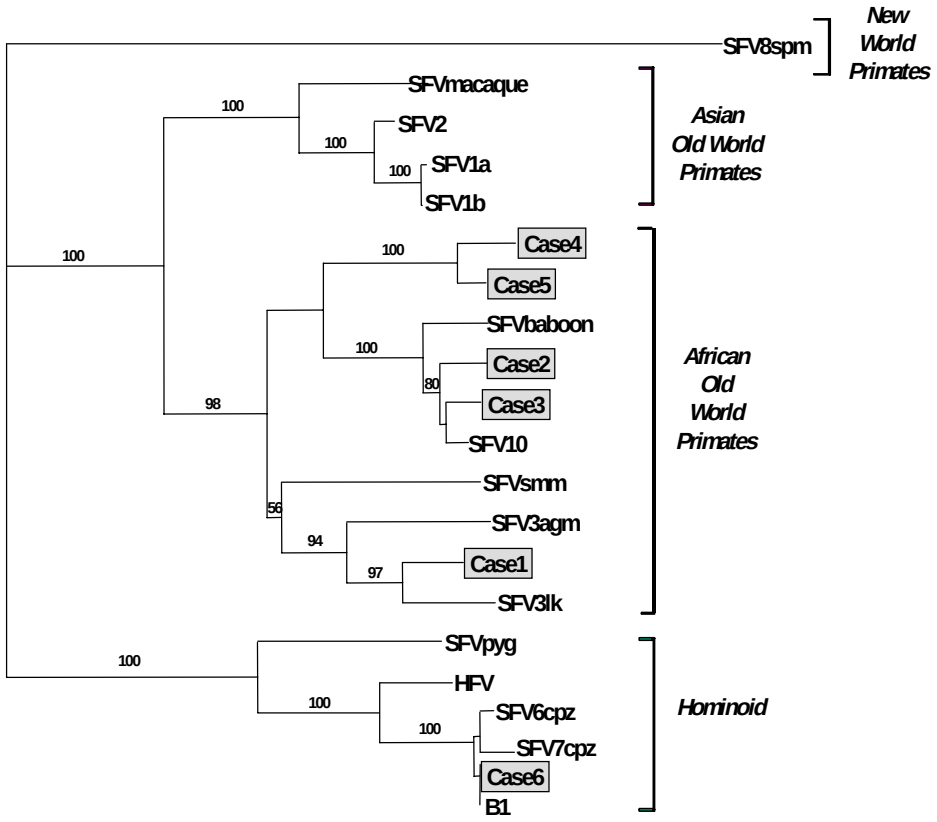
- Provided consultation to state and local health departments, clinicians, and concerned members of the public on the significance and appropriate clinical response to exposure to or infection with HTLV-I and –II.

Zoonotic Retroviruses and Xenotransplantation Activities

- Initiated a study of the phylogeny of simian foamy virus (SFV) in a large number of non-human primate species. This study will provide unique data which will allow precise identification of the origin of SFV found in humans.

- Identified seven additional persons with SFV infection through a survey of simian retroviruses among workers occupationally exposed to nonhuman primates (NHPs).

Cross-species Transmission of Simian Foamy Virus in Persons Occupationally Exposed to Nonhuman Primates



Neighbor-Joining Tree (SFV Integrase (425 bp))

- Began a lookback study on recipients of blood components from one SFV-infected donor.

- Published the results of the study demonstrating a prevalence of seroreactivity to SFV among zoo workers exposed to NHPs comparable to that found among workers exposed to NHPs in other occupational settings.

- Completed a study on the prevalence of seroreactivity to feline foamy virus, feline leukemia virus, and feline immunodeficiency virus among persons occupationally exposed to cats. No evidence of cross-species transmission was found

- Continued follow-up investigation of an SIV-infected laboratory worker. This person remains healthy, with no evidence of immunosuppression, 10 years after seroconversion. This person remains seropositive but has undetectable proviral and plasma virus loads.

- In collaboration with Circe Biomedical, continued to evaluate the risks of porcine endogenous retrovirus (PERV) transmission following exposure to porcine hepatocytes.

- Completed a study of the evolution of PERV sequences in species related to pigs. Data demonstrate that PERV has integrated in the pig genome before speciation.
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- In collaboration with Novartis Pharmaceuticals, completed a benchmark study looking for evidence of PERV infection in 160 persons exposed to living pig tissue through a wide variety of therapeutic modalities. Identified no evidence of PERV infection in any exposed persons. However, demonstrated persistent presence of microchimeric pig cells in the humans for up to 8 years after a transient exposure to a pig xenotransplantation product. In addition, this collaboration provided the most valuable information to date on the relative interpretation of results obtained using a variety of investigational molecular and serologic assays. This information has provided the most significant scientific basis for policy development to date in this field.

- Analyzed the susceptibility of PERV to licensed RT and protease inhibitors. Only AZT was found to be active on PERV. The data do not support the utility of the non-AZT drugs as prophylactic agents or for treatment of PERV-infected persons, if such persons are detected.

- Completed the development and approval process and began enrollment of eligible persons for a protocol providing an infrastructure for long-term follow up of persons infected with animal retroviruses. This protocol will create a framework for clinical, immunological, and virological evaluation and characterization of these persons, as well as evaluation of their household contacts for evidence of infection, and for long-term follow up of any persons identified as secondarily infected.

- Continued to investigate the source of RT activity in measles mumps rubella (MMR)

vaccines. Completed the identification of the types of endogenous avian leukosis virus (ALV) loci present in one cell substrate preparation. Evidence of loci that express both defective and nondefective ALV was found. Molecularly analyzed many loci, and developed locus-specific diagnostic assays for screening vaccines.

- Completed a study investigating transmission of endogenous ALV and endogenous avian retrovirus (EAV) to recipients of MMR vaccines. No molecular or serologic evidence of either ALV or EAV infection was seen. The data support current immunization policies.

- In collaboration with other members of the DHHS Interagency Working Group on Xenotransplantation,

  --analyzed and refined the systems development for the pilot National Xenotransplantation Database and began preparatory work to develop funding for and expand this into a national system.

  -- completed and cleared a revised PHS Guideline on Infectious Disease Issues in Xenotransplantation.

  -- completed and received final approval for a charter for a Secretary's Advisory Committee on Xenotransplantation (SACX) and solicited nominations for membership.

- Provided consultation on the development of appropriate public policy on xenotransplantation to the United Kingdom Xenotransplantation Interim Regulatory Authority (July 1999), the Swedish Committee on Xenotransplantation (March 1999), and Hannover Medical School in Germany (February 1999).

Immunobiology of HIV Infection

- Completed data collection of the pilot phase of the collaborative study with HIP/NCID and the Decatur Veteran Affairs Medical Center (VAMC) to longitudinally assess the relationships among intracellular cytokine patterns, HIV viral titers, CD4 cell counts, and other immune markers and the clinical course of HIV infection both before and during antiretroviral therapy.

- Completed studies of the effects of HIV infection and antigens and of the modulatory effects of anti-CD3 and mitogens on CD3, CD4, and CD8 expression.

- Completed studies showing that rapid progression of HIV disease is associated with adaptation to multiple coreceptor use, whereas persons with non-progressing disease (non-progressors) maintain exclusive R5 usage. We also have shown acquisition of elongation in V2 sequence among long-term non-progressors (LTNPS). The biological role of this acquisition is under investigation. Began research to examine the

polymorphism in the chemokine genes and their receptors.

- Studied prognostic markers for HIV progression. Published a paper on the SDF-1 gene variant and its correlation with HIV-1 progression. Collaborated with investigators in the Retroviral Genetics Laboratory, Center for Virus Research, Westmead Institutes for Health Research, Westmead, NSW, Sydney, Australia, on the evolution of of HIV-1 quasispecies in a longitudinal cohort of American patients with different disease progression rates: extended V2 region with acquisition of N-linked glycosylation site was uniquely present in slow and long-term non-progressors. Continued a study of HIV-associated lymphadenopathy in a cohort of homosexual men. This cohort study, which began in 1982, has examined prognostic factors for HIV progression.

- Developed fluorescence-based quantitative assay for HIV viral RNA measurement and gene expression assays for human RANTES and interferon gamma.

- Developed fluorescence-based quantitative assay to measure human thymic output.

- Completed studies to examine the expression of HIV coreceptors, CXCR4 and CCR5, on lymphocyte subpopulations in HIV-positive and -negative individuals and in persons before and after receiving highly active antiretroviral therapy (HAART).

- Completed studies of functional response and receptor expression in lymphocyte subpopulations in response to activation and to HIV infection.

- Completed study of CD4 T-cell regeneration in patients undergoing triple-drug antiretroviral therapy. Defined three subsets of CD4 cells with different trafficking and turnover patterns.

HIV Immune Response

- Examined a region of the HIV long terminal repeat (LTR) with homology to a kno murine HIV superantigen (SAG) and highly T-cell immunogenic for SAG activity.

- Evaluated serologic response in vaccine recipients from NIH cohorts (n=297) and the VaxGen phase II trial (n=~150) using several commercial tests and in-house gp41 peptide EIA to distinguish vaccinees from infected people.

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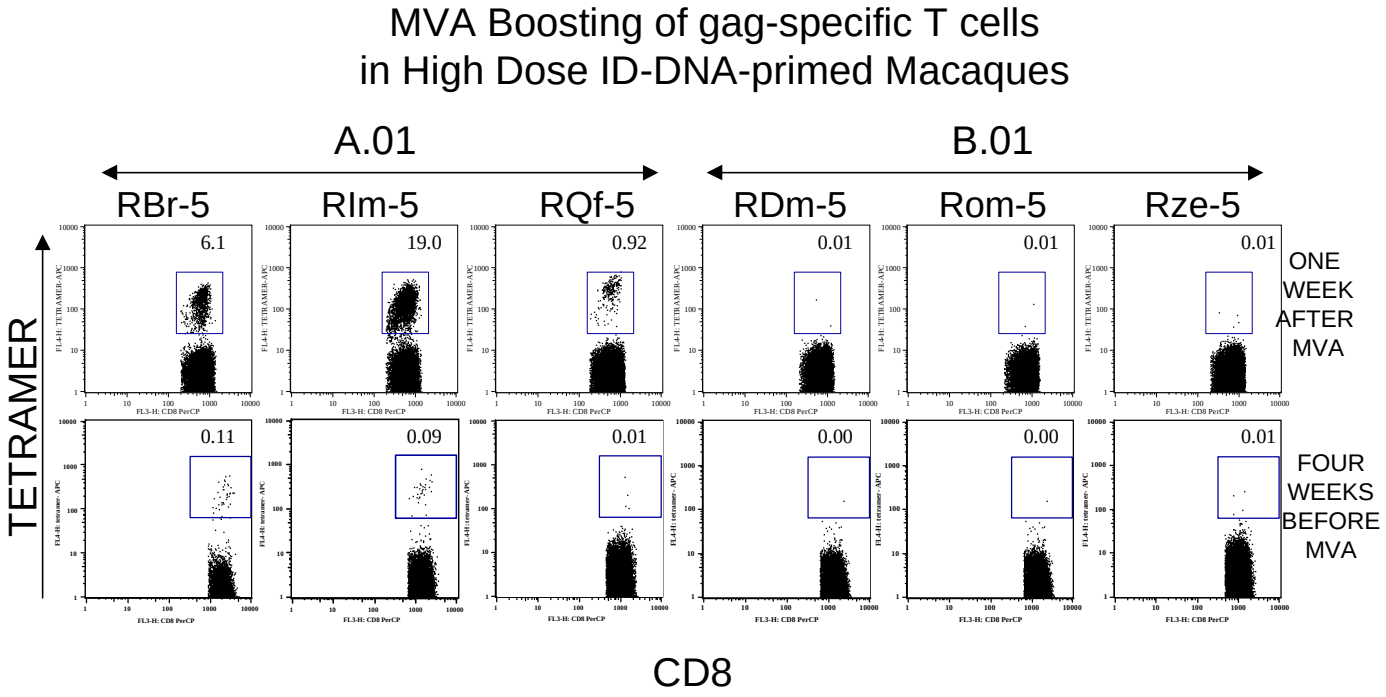
- Collaborated with investigators in the HIV Infection Branch, HIP, in a multicenter study of health-care workers exposed to HIV or other agents to determine whether any immunologic response to HIV occurs following such exposures.

- Initiated a trial of SHIV DNA/MVA vaccine in macaques at Yerkes Regional Primate Center at Emory University and CDC. Both vaccine components are immunogenic. Challenge vaccines with live SHIV will be given in this spring or summer.



HIV -associated Infections and/or Immunologic Diseases

- Assessed the relationships between natural T cells (NT), natural killer cells (NK), and γδ T cells and clinical mycobacterial, salmonella, and HIV infections. Also examined the relationship between NT and γδ. Manuscript submitted for publication.

- Conducted ongoing longitudinal study of idiopathic CD4+ T-lymphocytopenia (ICL). Provided consultation to physicians of ICL patients in the United States.

- Collaborated with the Viral Exanthems and Herpesvirus Branch, Division of Viral and Rickettsial Diseases (DVRD), NCID, and investigators at Emory University School of Medicine in studies of a new herpesvirus (human herpesvirus 8 [HHV-8]) associated with Kaposi's sarcoma. Participated in the evaluation of serologic and PCR-based tests for the virus Concluded a retrospective study of infection with HHV-8 and the course of the infection in a cohort of HIV-infected men who have sex with men in Atlanta.

Diagnostic Assay Development and Evaluation

- Streamlined routine diagnostic HIV serologic testing by fully implementing computerized data management system. Tested more than 15,000 specimens for antibodies to HIV-1, HIV-2, SIV, and HTLV-I or II infections.

- Continued to provide immunophenotyping for domestic and international studies.

- Evaluated multiplex assay and found results to be reproducible for 8 cytokines in supernatant samples but somewhat less reproducible for 7 cytokines in serum samples.
- Examined alternative strategies, based on antibody titers to specific viral proteins/peptides, antibody avidity/affinity, and proportion of HIV-specific antibodies, to develop a new assay to detect recent HIV-1 infection. A manuscript describing initial results is in preparation.

- Evaluated the detuned assay using longitudinal specimens from infants born to seropositive mothers (New York cohort) to assess its possible use for early diagnosis. Results were compared to IgG-Capture EIA. Additional specimens from the Pediatric AIDS Clinical Trial Studies (PACTS) will be tested.

- Initiated project to develop in-house *qualitative* HIV-1 RNA/DNA assay to be used for diagnosis in adults/infants and distinguishing vaccinees from infected persons.

- Assisted in the field evaluation of several rapid tests for their potential use in voluntary counseling and testing centers. Submitted a paper describing performance of several rapid tests on sera from people infected with different subtypes. Evaluated a pooling strategy for a rapid test (SeroStrip HIV-1/2) which should be very useful in developing countries.

Immunogenetics

- Coordinated analysis of CCR genes and analysis or testing of HLA genes in the HIV Epidemology and Research Study (HERS; 1300 genotypes completed), PACTS (69 genotypes completed), and CONRAD study (68 genotypes).

- Presented work on the role of CCR5 role in HIV transmission susceptibility (Chatauqua study) at the annual meeting of Genetics in Public Health and Disease Prevention.

- Completed initial studies of HLA and TNF genes with leptospirosis and observed risk of disease linked to several HLA DO or DQ types. Continued to optimize algorithms to study the structure and function of HLA molecules in HIV and arthritis. Continued HLA study of genetic susceptibility to rheumatoid arthritis after exposure to hepatitis B vaccine.

- Described and published new SNP (single nucleotide polymorphisms) and described new

IL-6 promotor haplotypes in African Americans. Observed that another IL-6 promotor polymorphism protects strongly against rheumatoid arthritis disease severity in African Americans. Continued studies of other genes in African Americans with rheumatoid arthritis.

- Established protocol for determining three polymorphic loci associated with the N-acetyl transferase gene (NAT-2), and typed 100 individuals. Established protocol for determining three polymorpic loci associated with the Vitamin D receptor

- Established HLA genotyping by DNA sequencing for determining HLA type for rare and unknown class I alleles

- Initiated studies to identify host genes required by HIV for replication.

Plans for 2000

HIV Biology, Molecular Epidemiology, and Development of New Surveillance Tools

- In collaboration with the Prevention Services Research Branch, DHAP-SE/NCHSTP, establish a unique database of incident strains of HIV-1 from 10 U.S. cities to help interpret breakthrough virus sequences from U.S. subtype B vaccine trials.

- Continue to study the evolution of mutation rates for HIV-1 and investigate whether there are differences in the mutation rates for different viral clades and if these rates change over the course of disease within a single individual or between individuals with different rates of disease progression.

- Develop rapid and sensitive detection assays for HIV activation from latency, and apply these assays to understanding HIV latency set-point and the impact of vaccination.

- In association with Coulter Immunology, Inc., explore HIV p24 antigen-capture technologies with and without acid-dissociation to identify incident infections in cross-sectional studies.

- In collaboration with DHAP-SE/NCHSTP and Yale University, continue to investigate female-to-female HIV transmission.

- In collaboration with the Surveillance Branch, DHAP-SE/NCHSTP, continue to screen persons in the United States with AIDS who have recently arrived from Africa in order to monitor the introduction and spread of non-B HIV-1 subtypes into this country.

- Develop new sets of primers for the efficient PCR amplification of the C2V3 region from the U.S. subtype B viruses.

Factors Affecting HIV Replication/AIDS Progression: Host Factors, Coreceptors, and Opportunistic Infections

- Examine host innate mucosal factors that may affect sexual transmission of HIV.

- Continue to examine the impact of various copathogens (*M. avium, M. tuberculosis*, *Cryptococcus neoformans*) on HIV replication and transmission using both the resting T-cell model infection system and the epithelial cell model system.

- In collaboration with the Hepatitis Branch, DVRD/NCID, and Johns Hopkins University, examine the influence of HIV on hepatitis C virus (HCV) load and disease.

- In collaboration with the Hepatitis Branch, DVRD, and the New York Academy of Medicine, study the impact of HAART on the natural history of HCV.

- In collaboration with the Tuberculosis/Mycobacteriology Branch, DASTLR, explore the impact of HIV infection on tuberculosis granuloma formation and determine the mechanisms of direct cell killing, cytokine dysregulation, and inappropriate adhesion molecule expression.

- In collaboration with the University of California at Los Angeles (UCLA) and utilizing the SCID-thy mouse model, examine the contribution of host trafficking molecules incorporated into the HIV envelope in viral dissemination to immune compartments.

- Explore host molecule incorporation into virus-like particles in which defined viral gene products have been deleted. Use this knowledge to better define vaccine candidates and begin to understand viral determinants that selectively incorporate or exclude host molecules in assembled virions.

- Continue to use the established mucosal infection protocol in the HIV-2 animal (*Macaca nemestrina*) model for a variety of applications, including studying vaccine-induced protection and microbicide protection.

- In collaboration with DHAP-SE/NCHSTP, use the HIV-2 animal model to study semen viral load during acute infection initiated via intravenous or rectal inoculation. Investigate transmission issues related to the impact of effective antiretroviral therapy and related to semen as an enhancing medium for either cell-free or cellular HIV transmission.

- In collaboration with BSTD and the Division of Parasitic Diseases (DPD), NCID, further apply mucosal models of HIV transmission to mimic sexual transmission and the impact of various STDs on mucosal transmission of HIV.

- In collaboration with UCLA and Antibody Systems, Inc., begin development of a foamy

virus vector for use in HIV vaccine application.

- Evaluate potential selective differences in viral phenotype that may arise when HIV is grown in cervical and/or intestinal immune cells in contrast to peripheral blood lymphocytes.

- Using the *in vitro* mucosal model, study differences in semen-derived HIV isolates in contrast to peripheral blood-derived HIV isolates to determine transmission differences.

- Complete refinement of the HIV-2/SIV quantitation assay in collaboration with Roche Molecular Systems under the established collaborative agreement for the accurate quantitation of HIV-2 field isolates.

- Begin analysis of additional cohorts of highly HIV-1 exposed, uninfected women, including exposed health-care workers, for cellular host factors capable of preventing HIV transmission. Explore viral-related factors and viral gene products for defects that might account for poor transmissibility.

- Continue to characterize the cellular pathways involved in drug inhibition of HIV transcription and the essential components and substrates involved in regulation. Explore mechanisms of drug selectivity and toxicity and applications in therapeutic intervention for activation from latency.

- Continue an examination of the mutation rate of HIV-1 from patients as a predictor of disease progression and development of drug resistance upon therapeutic intervention during acute infection.

- In collaboration with the AIDS Research Consortium of Atlanta (ARCA), the Decatur Veterans Affairs Medical Center, and the Viral Exanthems and Herpesviruses Branch, DVRD, complete analysis of the interaction of herpes virus infections with HIV-1 RNA plasma load in persons infected with HIV-1 who have CD4 counts >500/µl.

Emory Vaginal Ecology Study

- Continue prospective analysis of genital tract virus in HIV-1 infected women.

- Determine the replication kinetics of HIV-1 in the female genital tract.

- Expand cytokine and chemokine analysis and their relation to HIV-1 virus load in female genital secretions.

- Continue in situ hybridization studies for HIV-1 RNA to identify virus-producing cells in female genital secretions.

- Evaluate female genital secretions for HIV-1-specific neutralizing antibodies, and compare findings to the neutralizing antibody activity in blood.

- Investigate the relationship between genital tract levels of HIV-1 RNA and replication-competent virus using virus load and Amp-RT assays.

- Begin development of protocols to test in vitro effectiveness of vaginal microbicides against HIV-1 in vaginal secretions.

HIV Drug-Resistance Studies

- Expand development of rapid Amp-RT-based phenotypic assays to newly licensed drugs (e.g., efavirenz).

- Continue to provide support to the NCHSTP HIV resistance surveillance program through drug resistance testing and data interpretation.

- Apply rapid phenotypic testing for screening for nevirapine resistance in pregnant women and infants who have received nevirapine-containing prophylactic regimens.

- Identify differences in drug resistance in virus from different body compartments. This study will help assess whether such differences exist and, if so, which compartments may be most suitable for sampling virus for drug resistance testing. These studies will focus on comparing markers of resistance in peripheral blood lymphocyte proviruses or macrophages in contrast to plasma HIV-1 RNA in newly infected persons.

- Evaluate alternative genotypic methods (e.g., point mutation assays) that have a lower threshold of detection for drug-resistant mutations in viral mixtures than do currently used sequencing methods. These assays may be better suited for surveillance of drug-naïve seroconvertors who harbor mixtures of wild-type and resistant viruses.

- Study genotypic and phenotypic markers of drug resistance in non-subtype B HIV-1 and in HIV-2. Information gained will facilitate interpretation of drug-resistance testing results for these viruses and will increase understanding of the role of subtype B HIV-1 mutations in drug resistance.

- Examine compartmentalization of resistant HIV harbored in macrophages or T-cells as it correlates to vertical transmission in women who show no evidence of peripheral virus resistant to ongoing prophylaxis.

- Continue isolation, expansion, and titration of field isolates from individuals who harbor HIV genotypes suggestive of drug resistance. Continue interactions with contractors to generate phenotypic drug-resistance data on the isolates.

HTLV Surveillance and Disease Associations

- In collaboration with FDA and the New York Blood Center, examine samples from New York City blood donors to look for the presence of HTLV tax sequences.

- Continue to provide responses to public inquiries regarding exposure to or infection with HTLV-I and –II as appropriate.

Zoonotic Retroviruses and Xenotransplantation Activities

- Complete study identifying seven additional persons with simian SFV infection from survey of simian retroviruses among workers occupationally-exposed to NHPs and phylogenetic analysis linking case 6 to a specific NHP source. Explore potential for a collaboration with the National Institute of Occupational Safety and Health (NIOSH) and one or more primate centers to study work practices and workers' exposure to biologic fluids in order to develop a scientific basis for worker protection practices with biohazards associated with NHPs.

- Analyze the genetic changes that may occur in SFV following cross-species transmission to humans. Examine tissue distribution and virus loads in SFV-infected humans.

- Expand SFV seroprevalence surveillance to non-occupationally-exposed populations.

- Complete the lookback study on recipients of blood components from one SFV-infected donor.

- Continue surveillance of simian retroviruses in occupationally exposed individuals.

- Complete enrollment of currently eligible persons and contacts for a protocol for long-term follow up of other persons infected with unusual retroviruses (SIV, SFV, or other zoonotic and xenogeneic retroviruses) for investigation of disease associations, immunologic profiles, and human-to-human transmission, including transmission through blood donation. Continue to enroll newly identified persons infected with unusual retroviruses, and begin drafting initial report of clinical profiles, including information on the presence of virus in various body fluids, with implications for secondary transmission among humans.

- In collaboration with investigators at Emory University, evaluate the pathogenicity of SIV-$_{hu}$ to newborn macaques.

- Complete survey for evidence of seroreactivity to feline retroviruses among persons occupationally exposed to cats. Prepare *MMWR* article describing ongoing laboratory investigations.

- In collaboration with investigators at the University of Pittsburgh, the University of California San Francisco, and the University of Kentucky, publish results of investigations showing no evidence of persistent chimerism and xenogeneic retroviral infection in an HIV-1-infected recipient of a baboon bone marrow xenograft.

- In collaboration with investigators at the University of Nebraska, the University of Wisconsin, Johns Hopkins University, and McGill University, publish results of investigations for evidence of persistent chimerism and xenogeneic retroviral infection in four survivors of hemoperfusion through porcine hepatic xenografts. No evidence of PERV infection was found, although transient seroreactivity was identified in one recipient.

- Continue to investigate the presence or absence of PERV infection in persons exposed to porcine xenotransplantation products, publish the results of these investigations, and assess the implication of the outcome of the studies for public health policy development.

- Complete study on the identification of PERV in porcine factor VIII, which is used for treating hemophiliacs.

- In collaboration with Dr. Leonard Bailey at Loma Linda University, investigate the presence of baboon endogenous retrovirus (BaEV) infection in an infant who had received a baboon cardiac xenograft.

- In collaboration with colleagues at FDA, NIH, and HRSA, begin expansion of the National Xenotransplantation Registry into a national data collection system.

- Revise *PHS Guideline on Infectious Disease Issues in Xenotransplantation*, to be released for public comment in the *Federal Register*, March 2000, and published as a final guideline in September 2000.

- In collaboration with the DHHS Interagency Working Group on Xenotransplantation, define membership for the Secretary Advisory Committee on Xenotransplantation and begin operations and develop plans for financing and developing a central Biological Specimen Archive and implement such plans.

- Provide an advisor to an International Working Group on the Ethics of Xenotransplantation Public Policy, sponsored by the Humans and Nature Program, The Hastings Center, Garrison, New York.

- Analyze the prevalence of both ALV and EAV particles in chick-cell derived vaccines from various vaccine manufacturers. Determine the type(s) of ALV (replication-competent or defective) found in ALV-positive vaccines.

- Expand ALV surveillance to vaccine recipients known to have been exposed to vaccines

containing nondefective exogenous type ALV.

Immunobiology of HIV Infection

- Submit for publication results of the pilot phase of the collaborative study with HIP/NCID and the Decatur VAMC to longitudinally assess the relationships among intracellular cytokine patterns, HIV viral titers, CD4 cell counts, and other immune markers and the clinical course of HIV infection both before and during antiretroviral therapy. Obtain 6-month blood samples on individuals in this study and extend evaluation to that point. Analyze additional data on NT, γδ, and NK cells on these patients.

- Examine functional relevance of V2 extension, and test the hypothesis that V2 extension prevents emergence of X4 variants, and hence low viremia.

- Examine if SI variants lead to rapid CD4 decline and faster disease progression. Test the role of soluble factors, such as chemokines and cytokines, in the emergence of SI variants. Continue to study the role of tat and other viral proteins in HIV-1 disease progression.

- Complete analysis of in vitro and in vivo studies of chemokine receptor expression and function and publish results.

- Define by cell separation and nucleic acid detection the virus burden in CD4 T cell subsets that we have found to show differential CD4 T cell turnover.

HIV Immune Response

- Publish manuscripts from murine studies, and continue macaque DNA HIV vaccine experiments at Yerkes Regional Primate Center and CDC.

- In collaboration with the ARCA, participate in a multicenter immunophenotyping study of HIV-positive individuals treated with subcutaneous IL-2 who will be vaccinated with common vaccines. The substudy is to determine changes in the immune system in response to the treatment and vaccination.

- Continue to evaluate and monitor serologic reactivity of vaccine recipients in conventional tests and devise testing algorithms for distinguishing vaccinated from infected subjects. An additional 106 vaccinee specimens (from NIH) will be also tested by several assays.

- Improve expression system, purify, and evaluate for activity the construct of CD4 and C3d (fragment of 3rd component of complement) we prepared for use as a potential HIV immunopotentiating agent.

HIV-associated Infections and/or Immunologic Diseases

- Determine the pathway of how HIV tat protein activates latent HHV8.

- Continue investigations into the immunology of HHV8 infection and its association with Kaposi's sarcoma and other diseases. Evaluate additional peptide-based serologic assays for the diagnosis of HHV-8 infection.

- Continue participation in the prospective longitudinal study of individuals with HHV-8 infection in collaboration with the Viral Exanthems and Herpesvirus Branch, DVRD/NCID. Set up quantitative PCR assay for HHV-8 in plasma/serum/cells. Evaluate PCR ELISA assay for HHV-8 not requiring radioisotopes for detection of PCR product. Evaluate risk factors for developing HHV-8 infection in men who have sex with men to determine routes of transmission. Explore cell-mediated immune response to HHV-8 utilizing proliferation to viral antigens and cytokine secretion patterns in cells of infected individuals.

- Continue investigation of ICL to further characterize this "experiment of nature" both to impact clinical care of patients with this condition as well as to use the comparison of ICL and HIV infection to increase our knowledge of the specific immunologic defects found in HIV infection.

- Examine immunologic changes in persons infected with SFVs and other simian retroviruses; develop in vitro assays to measure the immune response to these viruses.

- Evaluate the immune response to HCV in the setting of parenteral exposure. Set up assays for immune response to hepatitis C. In conjunction with HIP/NCID and the Hepatitis Branch, DVRD/NCID, develop a protocol for evaluating post-parenteral exposure treatment with interferon and ribavirin to prevent virus transmission.

Diagnostic Assay Development and Evaluation

- Continue to perform high quality services for HIV and HTLV diagnosis, immunophenotyping, and antinuclear antibody testing.

- Implement EIA automation which will be done following proper validation. The supplemental Western blot assay has been automated.

- Acquire and test additional specimens from known seroconvertors infected with non-B subtypes to evaluate the detuned assay. Continue to provide testing and training support for epidemiologic studies.

- Continue to develop and evaluate alternate and improved methods for detecting early seroconversion

- Continue development and evaluation of in-house nucleic acid amplification assay for flexible, economic, and high throughput diagnosis of HIV infection.

- Continue developing and improving HIV subtyping (both genotype and serotype) assays to support domestic and international studies. Viral nucleic acid sequence information and detuned assays will be closely evaluated and used to improve the peptide-based detuned assays.

- Continue developing and improving serologic and nucleic acid-based methods for HHV8 diagnostics.

Imunogenetics

- Continue studies of CCR and HLA genes in HERS and PACTS. Refine HLA associations with HIV outcomes in the Multicenter AIDS Cohort Study (MACS) and test independently by applying to HIV-infected populations from Botswana in collaboration with R. Kaslow. Continue studies on host genes and TB in Botswana and in domestic TB transmission. Continue studies on HLA and other genes in persons with rheumatoid arthritis and leptospirosis. Publish new finding on recombinant break point in a multi-case arthritis family. Publish findings on DR4 and IL-6 in African Americans with rheumatoid arthritis.

- Develop and use gene-chip technology to quantify human and HIV gene expression.

- Identify human genes with altered expression levels upon HIV infection. Expression patterns of some of these genes may be useful in serving as markers for disease progression.